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FUNCTIONAL ASSESSMENT
OF
LASER IRRADIATION

ANNUAL PROGRESS REPORT

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Permanent functional alterations in visual acuity were observed with laser exposures (HeNe, Krypton and Argon) at corneal irradiance levels below those previously reported using fundoscopic or fine histological criteria. The effects of low-level irradiation appeared selective depending upon the wavelength of the exposing source and appeared to be cumulative over time. Monochromatic as opposed to achromatic targets appeared more sensitive measures of minimal functional disruptions.

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Introduction

Intense irradiation of the eye with coherent light not only alters retina morphology but also decreases visual performance. If the irradiation level is of relatively short duration ($<$100 mscc) and involves a significant portion of the fovea, both normal color vision and maximum visual acuity are affected.

Historically, the maximum permissible exposure (MPE) to laser light has been based purely on morphological criteria. Morphological assessments have included gross fundoscopic examination of the eye following acute exposure to intense irradiation as well as more sophisticated examination of the cellular layers within isolated sections of the exposed retina. These morphological techniques have provided information as to the specific site of primary damage as well as the energy levels involved in producing such structural alterations. Generally, however, single exposure of relatively long duration and large diameter on the retina have been employed to determine the MPE. While these exposure conditions may facilitate visual verification of the induced alteration they do not correspond to the type of conditions under which accidental exposure may occur in the field. Two relatively different hazards exist with the employment of lasers. First is either the accidental or intentional exposure to an intense but brief laser beam in the field. Associated with this type of exposure would be a relatively small area of the retina. The second type of exposure is the intentional viewing of presumably safe and low level diffuse irradiation over relatively long time periods: days, weeks, or even months.

While morphological data have greatly contributed to the ANSI (American National Standards Institute) guideline they provide no data regarding the degradation in visual performance following exposure near to or above the MPE. Such information is critical when discussing the possibility of completing a visually-guided mission by a person exposed either accidently or intentionally to an intense, short duration laser flash. Further, morphological criteria alone may produce safety standards above those derived using other types of assessments. Both behavioral and electrophysiological criteria of light-induced retina alterations may reduce significantly the MPE since minute biochemical and/or structural alterations association with laser exposure are often difficult or impossible to identify.

In most behavioral studies, like morphological studies, anesthesia is usually required for placement of specific retina lesions; the exception being those studies where the entire retina is chronically exposed to diffuse laser light. Anesthesia, however, eliminates all possibilities of immediate postexposure acuity measurements for at least 24 hoursand thus seriously limits the exploration of transient changes in acuity by this method. The immediate changes in visual performance are critical both in the exploration of minimal thresholds as well as testing for any cumulative effects of repeated exposures to energies below the MPE. In the initial phases of this contractual effort, a behavioral procedure was developed for producing consistent foveal retinal exposures in awake, task-oriented animals using relatively small retinal spot sizes. This procedure has been used along with a modification of a rapid method to measure rhesus visual acuity prior to and immediately following brief exposures to laser exposure.

In the original protocol, spot size of the laser beam on the retinal surface had to be restricted to less than 200 microns due to power limitations of conventional HeNe laser sources. During past project periods, Krypton and Argon lasers were used which had an increased power output over standard HeNe lasers. These laser systems allowed for increased spot sizes on the retina of sufficient power densities to produce functional alterations in visual performance. Further, the use of the Krypton laser with a spectral output (647.1 nm) not significantly different from the HeNe output (632.8 nm), allowed for a direct comparison of the effects of spot size on the magnitude and duration of the functional alteration without regard to the wavelength of the induced effects. The rationale for use of larger spot sizes (in excess of 300 microns diameter) is to make the results from these functional explorations more compatible with those of morphological studies underway in other laboratories. Large spot sizes facilitate histological exploration. In addition, the use of larger spot sizes in our functional studies allows for histopathological and electrophysiological examination of our subjects' retinas at Letterman Army Institute of Research following the subjects termination in the behavioral portion of this research program. A third advantage of larger spot sizes is that they increase the probability of a foveal exposure in any given session since a larger retinal area will be affected. In past studies we have not found that the subject typically could "look" around the affected retinal area and still make the required baseline "photopic" discriminations (i.e., resolve a target of 1.0 min^{-1} or less). If a deficit was elicited in acuity, both the magnitude and duration of the deficit suggested total foveal involvement occurred and any attempt to "look" around the affected area resulted not in foveal but rather parafoveal or peripheral vision. We are currently investigating along with investigators at Letterman Army Institute of Research any significant shifts in spectral sensitivity following laser exposure to also verify our assumptions of total foveal involvement. Larger spot sizes should also decrease the probability of a foveal "miss" and increase the magnitude of the acuity deficit elicited.

In the past, visual acuity recovery functions were derived using only achromatic targets. Since the area of primary disruption is in the fovea where color vision predominates, it is more appropriate to test for alterations in visual acuity using monochromatic targets rather than achromatic targets. The post-exposure spectral sensitivity curves derived should better delineate the overall consequences of laser exposure on visual performance and perhaps better delineate the damage mechanism. It is more than likely that certain types of receptor cells, based on the absorption spectra of their underlying photopigments, will be affected more severely by the specific wavelengths of laser irradiation than will other photoreceptors which have their peak absorption at opposite ends of the spectrum from the exposure light. Testing visual performance using white light backgrounds will not delineate specifically these spectral effects and will result in functional thresholds for permanent damage at a much higher energy level.

In the present study we have measured the immediate as well as long term recovery of visual acuity using both chromatic and achromatic test targets. The long term consequences of repeated low level exposures of HeNe, Krypton, and Argon laser light was also compared. Initial exposures are presented well below the MPE and the power density on the retina is then systematically increased in discrete steps over a period of time until the recovery process is no longer complete. Recovery functions are derived using a continuous assessment of the subject's acuity and continued until total recovery is maintained or, in the case of exposures above threshold, until the recovery process stabilizes. Several different assessments are then made of the resultant visual impairments. The goal of this project is to extrapolate both the threshold retinal power densities and deficits in visual performance from rhesus to human. The hypothesis is that receptor viability and recovery for the rhesus and human are similar and are only displaced along the energy dimension because of either differential pigmentation or retinal illumination for the same external physical source.

Methods

In a previous paper we have presented a method to expose awake, task-oriented rhesus monkeys (Robbins, Zwick, & Holst, 1974); a procedure necessary to record immediate changes in acuity following exposure. Visual acuity was measured using conventional black Landolt rings against achromatic (white) or chromatic backgrounds. Rhesus were trained to press a lever whenever a Landolt _C_ was presented and not to respond when gapless rings were presented. If the subject failed to respond to a Landolt _C_ during the 2 sec presentation, or if he responded to a gapless ring, he received a brief electric shock which was annoying but not highly painful or dangerous. Landolt _C_'s were randomly arranged within a series of equally-sized, gapless rings. Threshold acuity measurements were obtained by a tracking method which allowed the subject to adjust the size of the test object about his threshold. All testing was performed monocularly under photopic conditions. Chromatic backgrounds were equated for equal numbers of quanta.

Several different lasers (HeNe - 632.8 nm; Krypton - 647.1 nm; and Argon 614.5 nm) have served as exposure sources. The diameter of the beam on the retina was varied between 150 and 323 microns depending upon exposure conditions. The beam was aligned such that it was coaxial with a line between the artificial pupil and the gap in a specified Landolt ring subtending less than 1 min of arc. The beam passed through a converging lens positioned such that the cornea was in the focal plane of the lens. The animals' head was also rigidly held in a fixed position and fitted with an opaque facemask and monocular iris diaphragm so that eye position could be well controlled during testing. These restraints minimized the effects of pupillary changes and lateral head or eye movements and resulted in an increased probability of foveal exposures (75%). Exposures of 100 msec duration were made only during threshold discriminations and were triggered by the animal's correct detection of his threshold Landolt ring. Exposures were made over power levels from 0.3 mW to 11 mW measured at the cornea, beginning with the lowest power level. No more than one exposure was made per day and each power level was repeated a minimum of 4 times for each exposure condition. Immediately after exposure the recovery of acuity was measured using both achromatic and chromatic background targets. If the subject failed to return to his preexposure acuity level within the 2 hr test session, further exposures on subsequent days were suspended and daily baseline measures of spectral and white light acuity were obtained.

Results

Sample data of threshold acuity using the tracking technique is shown in Figure 1. In this session the subject was exposed to a 7 mW, 150 micron HeNe flash of 100 msec duration. The occurrence of the exposure is indicated in the figure by an arrow (zero point on the abscissa). The ordinate indicates the various sizes of the gaps in presented Landolt rings and is plotted in reciprocal minutes of arc. The order of presentation was dependent upon the subject's response on Landolt ring trials. Incorrect detection of the Landolt C caused the recorder to plot downward and corresponded to the presentation of larger ring diameters. The abscissa represents the presentation of the Landolt Cs, and corresponding times (in minutes) for representative trials are indicated relative to exposure.

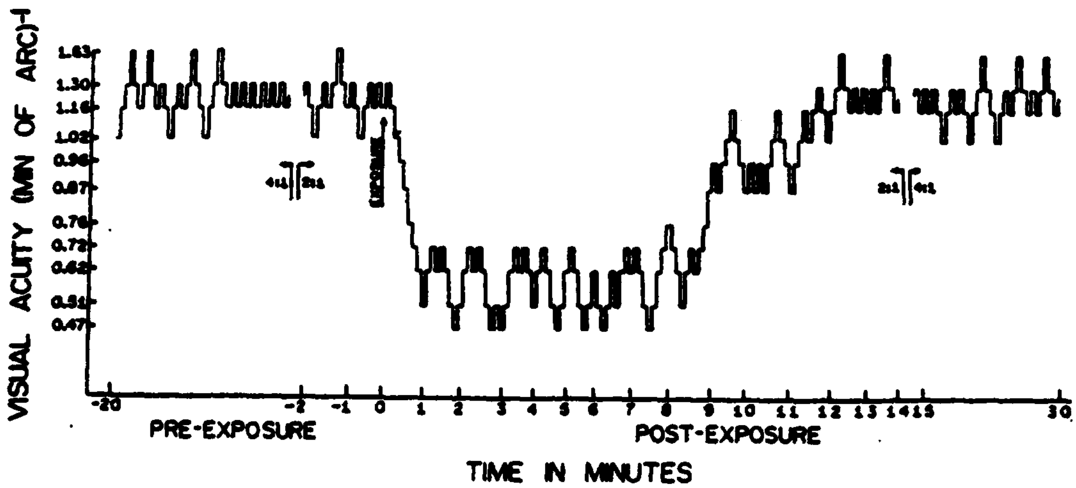

Figure 1. Raw data recovery function.

Prior to exposure, the subject's mean acuity under maximum photopic conditions was 1.25 (min of arc)$^{-1}$. Immediately following exposure the subject's acuity to achromatic targets decreased 59% to an acuity level of 0.51 (min of arc)$^{-1}$ and full recovery was complete within 13 minutes. The magnitude of the initial deficit in visual acuity following exposure was independent of exposure power. With a larger retinal spot size of 323 microns, a significantly greater initial deficit (69 to 86%) was produced representing an inactivation of a larger portion of the retinal mosaic. With chromatic targets the initial deficit in acuity varied from 0.40 (min of arc)$^{-1}$ for backgrounds of 560 nm to 0.20 (min of arc)$^{-1}$ for backgrounds of either 640 or 480 nm but the magnitudes of these deficits for any specific wavelength target was also independent of the energy of the flash.

Comparisons of the temporary effects of single-pulsed, laser exposures (647.1 nm, 1.0 mW, 100 msec) on visual performance under different monochromatic viewing conditions are shown in Figure 2. Visual performance was defined as the minimum perceived gap in a Landolt ring or threshold acuity determined for five different background wavelength conditions. All backgrounds were presented equated for equal energy and the subject adjusted the size of the gap in a black Landolt ring about his threshold level. Threshold was defined in the conventional way as that minimal gap size which was detected 50 percent of the time. While in the process of m. ing these threshold observations, the animal was exposed to a '7.1 nm flash that exposed a 324 micron spot centered on the fov. Each curve in this figure represents the mean of several expo.ures presented at a minimum of 24 hours apart. Plotted for each background wavelength is the mean percent deficit in visual acuity, relative to the pre-exposure level, as a function of time following a 1.0 mW laser exposure. Immediately after exposure the subject's acuity decreased to a maximum of between 70 to 80 percent of the pre-exposure level depending upon the wavelength of the test target. For the short wavelengths 480 nm to 520 nm) the initial deficit in visual acuity was greater than those observed for the intermediate and long wavelength regions of the visible spectrum. For the shortest presented background wavelength (480 nm), not only was the initial deficit the greatest but so also was the total time of recovery. Minimum initial deficits and the most rapid recovery times can be noted for the intermediate and long wavelength test targets.

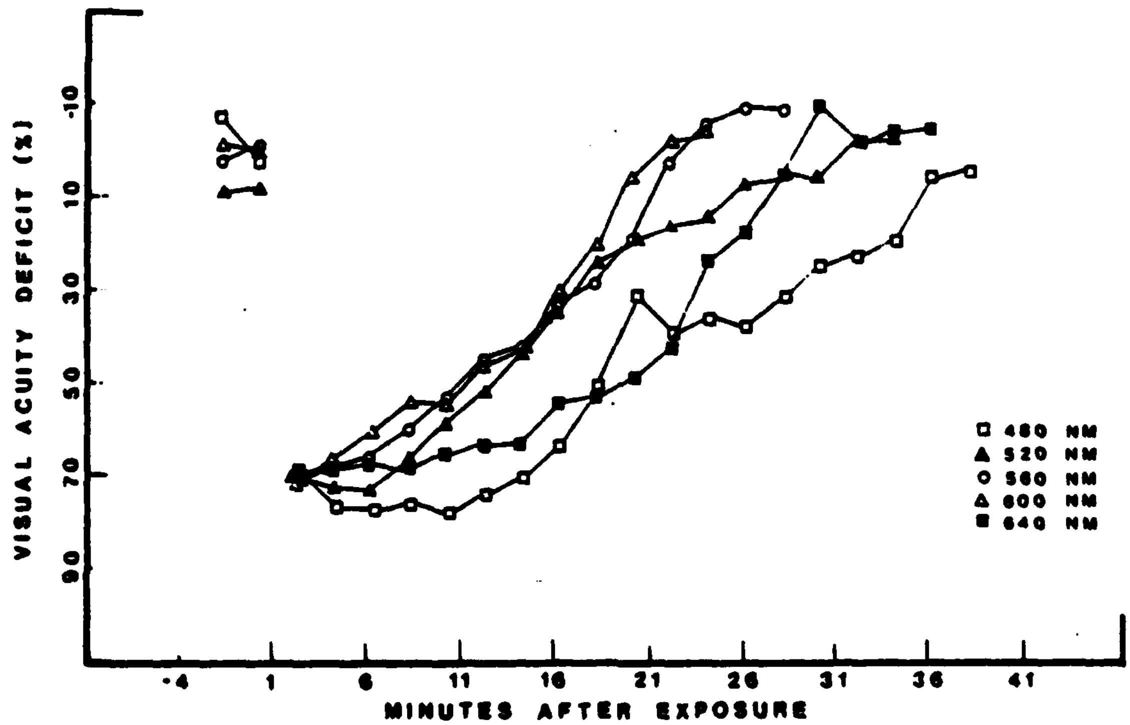

Figure 2. Laser recovery following a 1 mW exposure for different monochromatic targets.

Changes in the energy of the laser exposure produced little overall differences in the magnitude of the initial deficits but did produce significant changes in both the total time of recovery and the rate of recovery during the initial phases of the recovery process. Representative recovery functions for 560 nm test targets as presented in Figure 3 for a series of laser flashes beginning at 1.0 mW and ending at 6.0 mW. Similar to the curves shown in the previous figure, each curve in this figure represents the mean of several exposures at each of these different exposure powers. For the lower exposure energies, the total recovery was relatively fast and the slope of the recovery functions relatively steep. But as the exposure energy was increased the total time for complete recovery increased and the initial deficit remained depressed longer before recovery more gradually occurred with time.

A permanent functional deficit in visual acuity was elicited following the third exposure to a single-pulsed, 6.0 mW flash. The deficits in visual acuity following each of the three separate exposures are shown in Figure 4. Only one exposure was presented during a test session and the animal was first exposed, at this power level, to a 560 nm test target. For this wavelength and exposure, the subject's acuity returned to his pre-exposure baseline level in approximately 36 minutes but then again became depressed during continued testing and remained depressed until the end of the test session. Visual acuity was again tested the next day and for the following five days without any additional laser exposures and during this time visual acuity for various wavelength targets was consistently within one standard deviation before any exposures were presented to the subject). The second 6.0 mW flash was presented on the sixth day and recovery was followed using a 480 nm test target. After a slightly less initial deficit in comparison to the 560 nm test target, the subject's performance returned to its pre-exposure level more rapidly and full recovery was realized in 28 minutes. The subject's acuity was examined for an additional 30 minutes after full recovery and his acuity level remained within one standard deviation of his pre-exposure baseline level. The following day the subject was exposed to the third and final laser flash. The changes in visual performance immediately following exposure were assessed using a 640 nm test target. Initially, the deficit in visual acuity was much less in magnitude than those elicited following the order 6.0 mW exposures but this initial deficit remained depressed longer before the recovery process began. Once the recovery process did begin, full recovery (i.e., a return to pre-exposure baseline acuity levels) occurred quickly and was complete in approximately 33 minutes. Furthermore, the subject's threshold acuity continued to rise above his pre-exposure level and remained elevated, though variable, for the remainder of the test session. The subject's visual acuity, however the next day was significantly depressed and remained depressed for the next thirty days of post exposure testing. Spectral response curves were measured throughout this period and representative curves are shown in Figure 4. Pre-exposure acuity was maximum at 560 nm under the photopic test conditions employed in this study and acuity

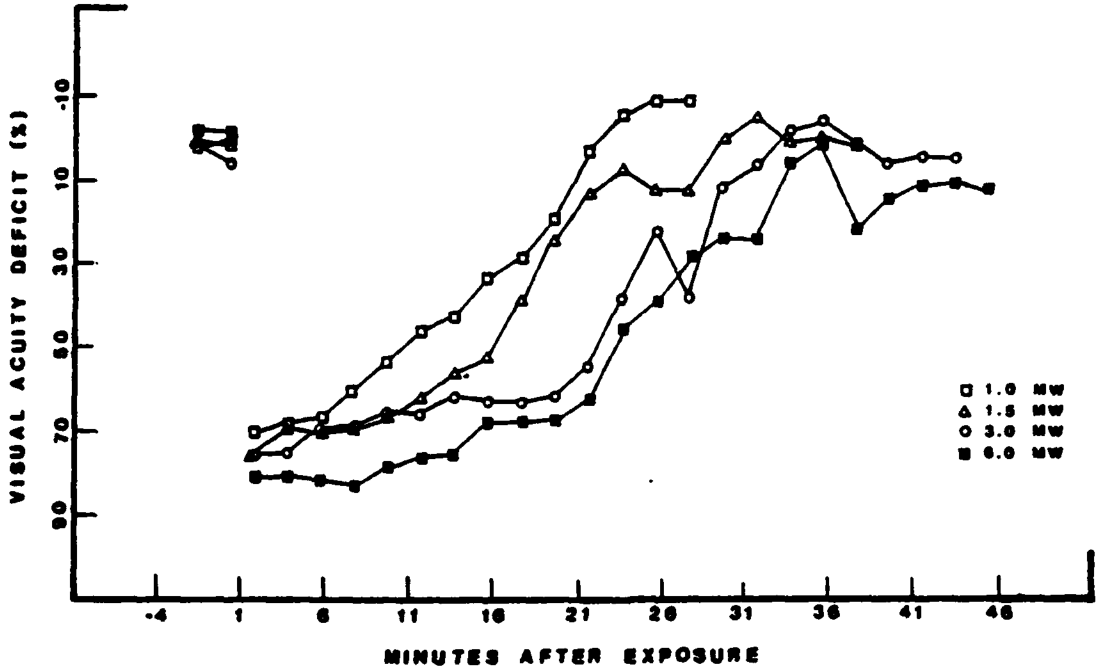

Figure 3. Laser recovery following various exposure energies

quickly decreased beyond 600 nm. Immediately after the third exposure to a single pulsed, flash and for the next five days, the entire acuity function was equally depressed across the visible spectrum. During the next several weeks some slight recovery was noted for 520 nm and 600 nm test targets although during the entire course of postexposure testing acuity measured with monochromatic backgrounds never returned to its pre-exposure level. Furthermore, the peak of the postexposure spectral response function shifted from a maximum at 560 nm to a maximum at 520 nm. No significant shift in pre- and postexposture acuity measured using white backgrounds or measured binocularly using monochromatic backgrounds was noted. Up until the animal's unexpected death, no significant recovery in spectral acuity had taken place and postexposure achromatic acuity remained at its pre-exposure level. Further postexposure testing would have been necessary to discount any long term recovery process.

These results obtained with the Krypton laser (647.1 nm) compare nicely with our previous data using a HeNe source (632.8 nm). With the 632.8 nm line our spot size on the retina was much smaller and likewise the magnitude of our initial deficit was smaller suggesting that the larger spot diameter of the 647.1 nm line brought into involvement more parafoveal cone receptors. Additionally, the threshold established for permanent functional damage in the HeNe study was 11.0 mW while in the Krypton study, threshold occurred at 6.0 mW. The difference

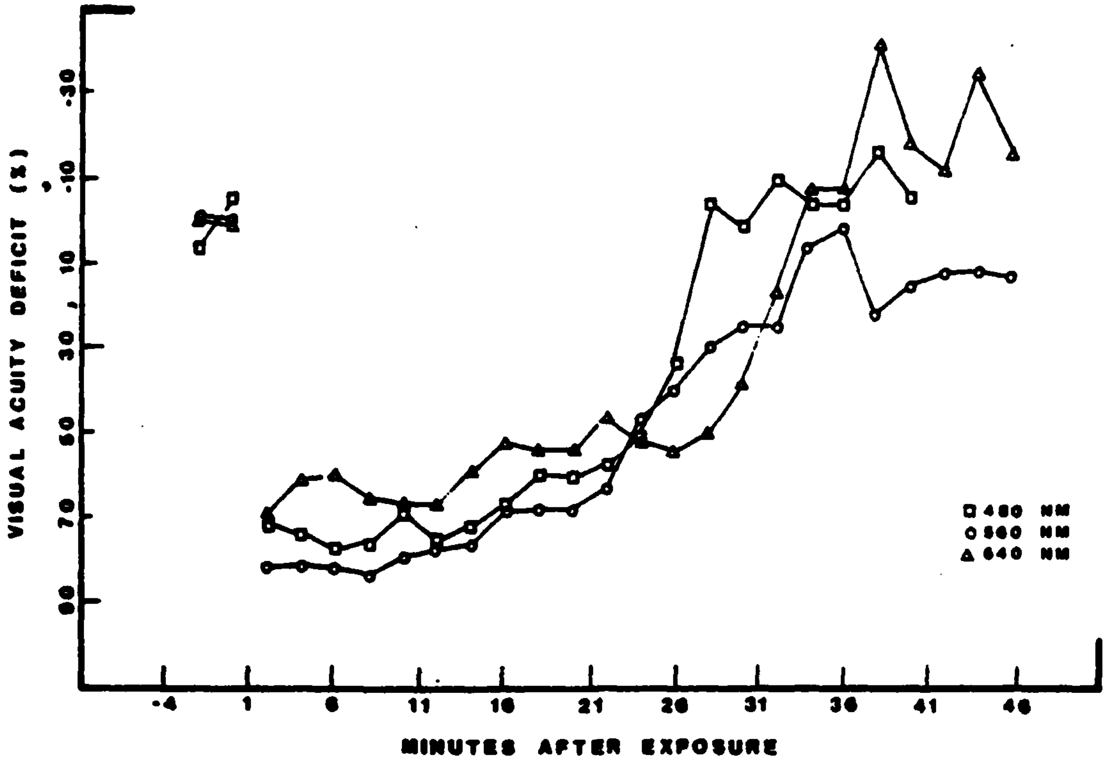

Figure 4. Laser recovery following a 6.0 mW exposure.

between these two points may be accounted for by the fact that monochromatic acuity targets are a more sensitive measure of foveal, hence cone, function than are white light acuity targets. Achromatic targets used during postexposure testing did not reveal any evidence of functional damage (i.e., postexposure acuity was the same as that observed during pre-exposure testing) although a significant depression in postexposure acuity was noted for monochromatic targets (see Figure 5).

One subject was exposed to a 514.5 nm (Argon) laser instead of the 647.1 nm laser used in the previous figures. All other exposure conditions were held constant. Pre-exposure and postexposure acuity measurements were made using both achromatic and monochromatic background targets. In Figure 6 the recovery functions for three separate 3.0 mW Argon exposures are shown. Postexposure acuity in this figure was measured using achromatic backgrounds. Similar to the deficits noted previously with Krypton exposures, acuity dropped immediately following exposure to approximately 60-80% of its pre-exposure level. The magnitude of this deficit was significantly greater than those produced by the smaller-diameter, HeNe exposures. Little differences were seen in the magnitude of the initial deficit for different exposure power densities. The background of the test target used to measure visual acuity also did not significantly affect the magnitude of the initial deficit although

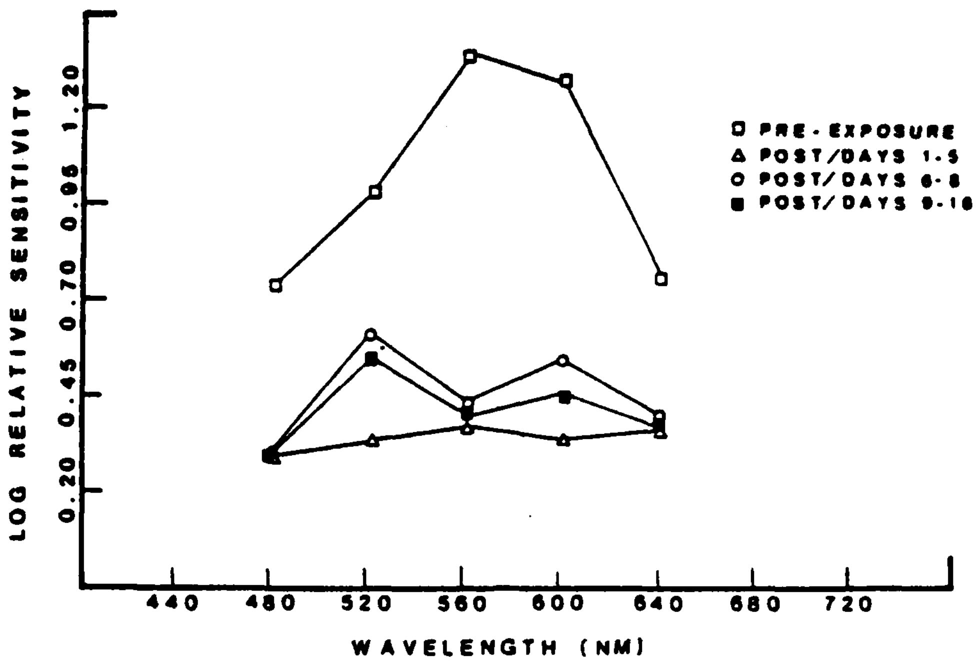

Figure 5. Spectral sensitivity of one rhesus monkey before and after laser exposure. The pre-exposure spectral sensitivity curve was measured over several weeks of baseline testing before laser exposure. The postexposure spectral sensitivity curves were measured following the third exposure to a 6.0 mW flash (100 msec) from a Krypton laser. Spot size on the retina was 324 microns.

generally short wavelength acuity was more affected both in terms of the magnitude and duration of the initial deficit than either intermediate or long wavelength acuity when 514.5 nm laser exposures were made. This differential susceptibility of a short wavelength process was not as evident when using Krypton exposures. In the top portion of this figure, the recovery function for the third exposure to 3.0 mW of Argon light is shown. Recovery was rather rapid and reminiscent of recovery functions derived at lower power levels for this and other wavelength background targets. The middle plot shows the recovery function for the seventh exposure at that power level and for similar achromatic background targets. In the lower plot, the ninth and final exposure over a 15 day period is shown for the 3.0 mW exposure level. Recovery after the ninth exposure level was slower but was complete approximately 15 minutes after exposure.

Twenty-four hours later, however, a significant deficit in both achromatic and chromatic acuity was noted. In Figure 7 pre-exposure and postexposure spectral response curves are shown for

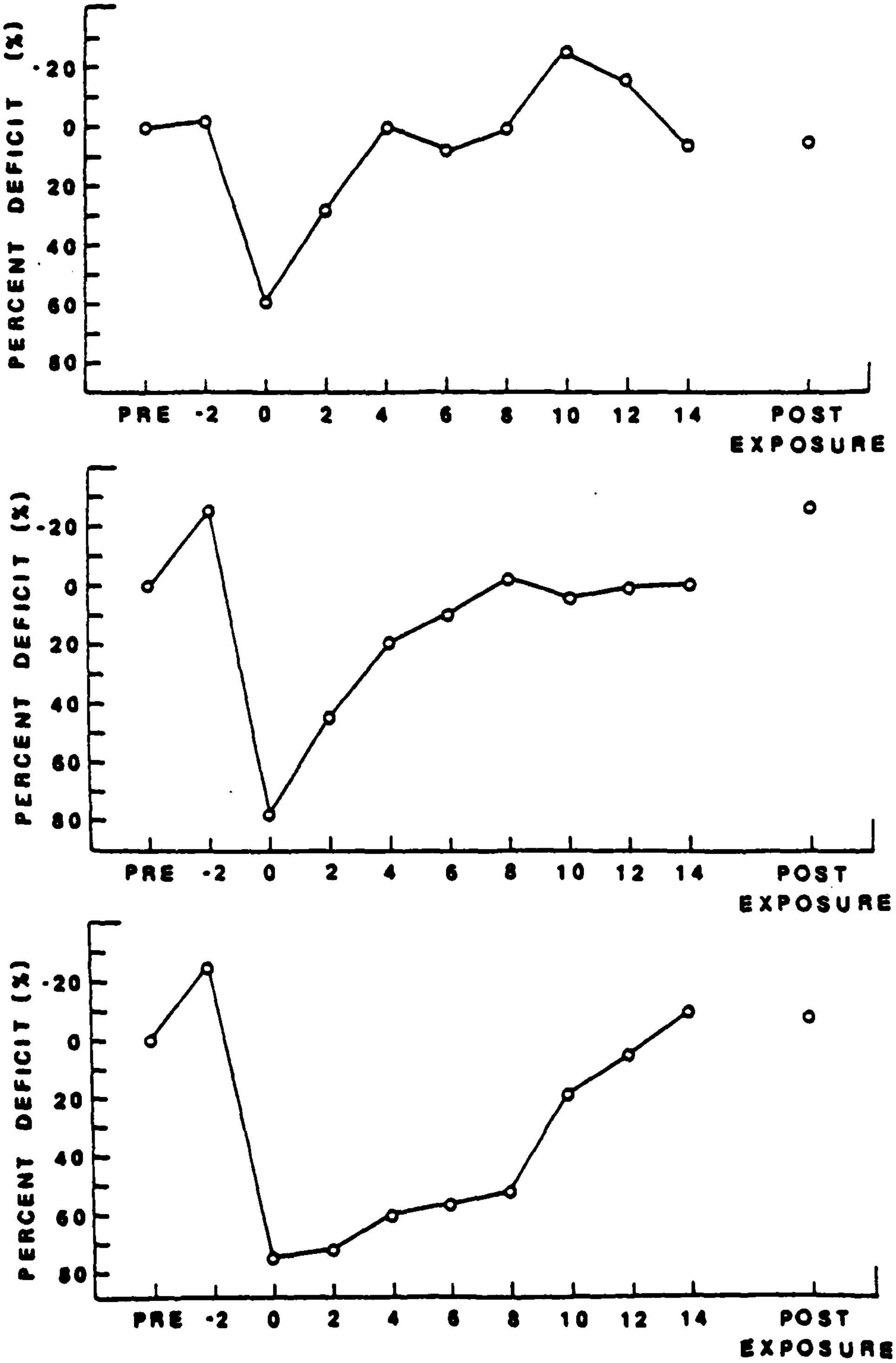

Figure 6 Recovery functions for one subject following single, 3.0 mW exposures on days 2, 6, and 9 to a 100 msec 323 spot of 514.5 nm laser light. The background of the test target was achromatic.

preselected background targets of different wavelengths equated for equal numbers of quanta. Generally, for the first five days postexposure, spectral acuity was uniformly depressed across the entire spectrum including achromatic background targets. Within two weeks of the last exposure, recovery to pre-exposure acuity had been accomplished for 560 nm background targets and to a lesser degree for 620 nm and white light (000) background targets.

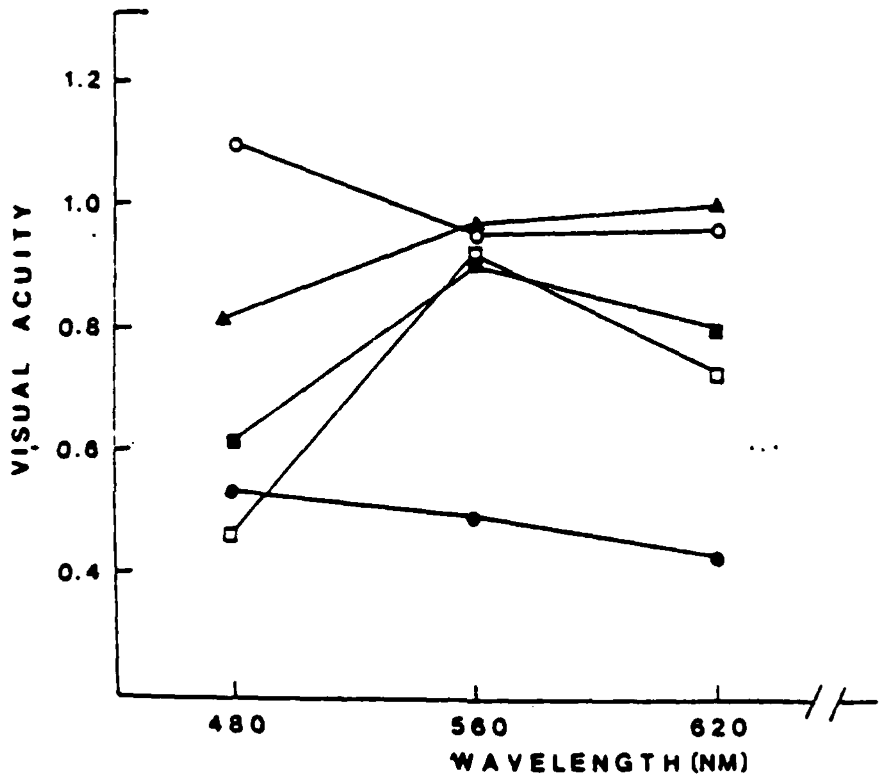

Figure 7. Pre- and postexposure spectral acuity for one subject following the ninth exposure to a 3.0 mW, 514.5 nm laser exposure. All data points represent the mean of several test sessions and all background wavelengths were equated for equal number of quanta.

No significant recovery to short wavelength targets, however, was seen one month after exposure. Almost two months after exposure spectral acuity in the exposed eye, relative to the control or unexposed eye, was still significantly depressed in the short wavelength region of the visible spectrum although at this time no significant long term deficits are evident in the intermediate or long wavelength region of the spectrum (see Figure 8). Likewise postexposure acuity to achromatic background targets returned to pre-exposure levels within 45 days of exposure.

This animal has not received any additional exposures to Argon irradiation. The subject's postexposure spectral acuity is continually being assessed to determine if any additional recovery will occur. During the next proposed contract period, the subject's postexposure spectral acuity will be examined on a weekly basis and depending upon the nature of any additional recovery, a decision will be made regarding further exposures at this or higher power levels.

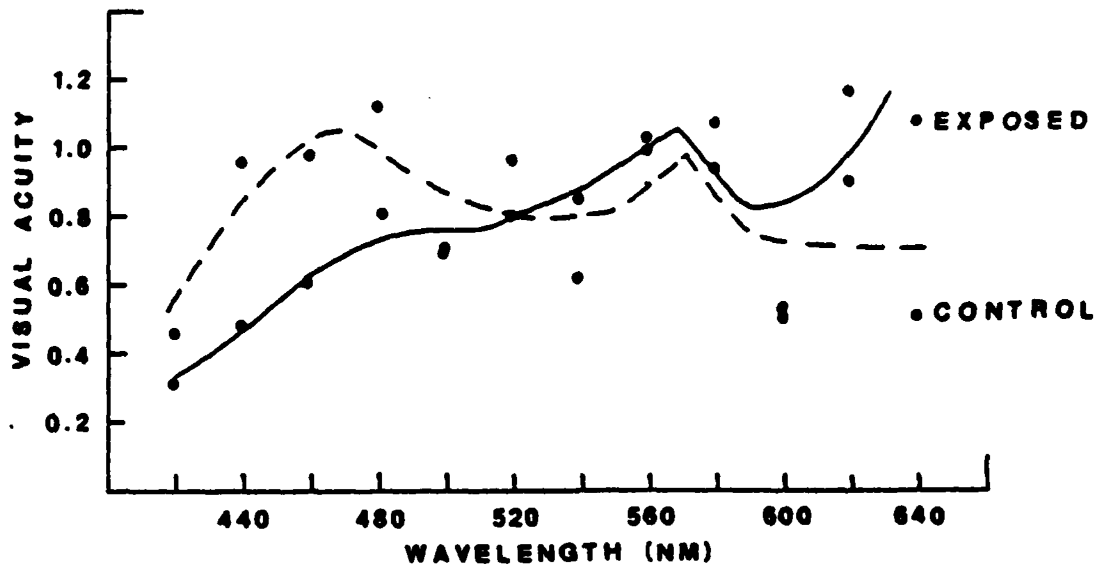

Figure 8. Postexposure spectral acuity in the exposed and control eye two months following the ninth exposure to an 3.0 mW, 514.5 nm flash.

Discussion

Permanent functional alterations associated with laser exposures occur at corneal irradiances below those previously reported using gross fundoscopic or fine histological criteria. Further, the effects of low-level irradiation appear selective depending upon the wavelength of the exposing source. With Krypton irradiation, the maximum transient deficits in acuity occurred for targets on long wavelength backgrounds and no permanent alterations were noted for achromatic acuity following irradiation levels which significantly depressed the entire chromatic acuity function. With Argon irradiation, the threshold for permanent functional alterations in chromatic acuity was lower than for either HeNe or Krypton exposures but no significant differences were found between thresholds when achromatic and chromatic targets were used. Both acuity functions were somewhat uniformly depressed for at least five days before recovery gradually occurred. After approximately two months the subject's postexposure acuity had returned to its pre-exposure level for all targets except those in the short wavelength region of the visible spectrum where a significant deficit still remained. These data suggest that specific spectral lines of coherent light at low levels selectively alter specific foveal cone processes and the use of chromatic acuity best delineates this effect.

In all of our studies, the transition from temporary to permanent losses in visual acuity occurred after a succession of exposures at the same cornea irradiances and not after the first exposure at any particular exposure level. This phenomenon occurred in spite of the fact that we waited a minimum of 24 hours between repeated exposures and longer if the subject had not fully recovered within the original 2 hour test session. Such an effect is strongly suggestive of some cumulative process occurring in the eye to repeated exposures. A similar result was shown when animals were exposed to daily low-level, diffuse Argon irradiation, using a different paradigm (Zwick, Bedell, and Bloom, 1974). These results are more closely analogous to reported temporary and permanent threshold shifts in audition than those reported for the visual system. No distinct long-term chemical process has yet been implicated within the eye in which changes of this nature could occur although other equally acceptable explanations could include changes in receptor or neural electro-chemical activity or structural changes either in the receptor cell, pigment epithelial cell or other accessory structures associated with light reception and transmission.

Several important conclusions can be drawn from our studies thus far which need further examination and delineation in the future contract periods. First, the effects of intense, single-pulsed, laser irradiation appears cumulative. In all our experiments, regardless of the exposure wavelength or the type of background target used to assess visual acuity, a permanent deficit in visual acuity was found not after the first exposure at a new power level but after multiple exposures at the same power level spaced a minimum of 24 hours apart. Second, monochromatic test targets appear to be a more sensitive measure of postexposure acuity than achromatic targets, especially for longer-wavelength laser exposures (HeNe and Krypton). Using Argon irradiation both achromatic and monochromatic acuity demonstrated a significant depression following relatively low level (3.0 mW) exposures although acuity to chromatic targets remained depressed longer than acuity to achromatic background targets.

Third, the power level where a permanent functional alteration was found appears to differ significantly depending upon the wavelength of the exposing source (6.0 mW for Krypton and 3.0 mW for Argon). Compounding the conclusion, however, is any cumulative nature of the damage mechanism; although, with both exposing lasers, animals were exposed on an almost identical daily basis to power levels of increasing power densities on the cornea.

These experiments raise questions as to the exact nature of the damage mechanism of acute laser irradiation. These data do, however, begin to delineate the adverse effects of laser irradiation on immediate as well as long term visual performance. No experiment or series of experiments can include all the necessary stimulus parameters to cover all of the possible situations where disruptions in visual performance might occur. Rather a series of key stimulus dimensions are being used to depict the underlying neurological and morphological mechanisms which alter visual performance and could disrupt successful completion of visually-guided missions. It is from the data of these types of experiments that we propose modifications in the existing safety standards or the establishment of new standards where none now exist as in the case of chronic exposures. As this and other projects have shown, the sole use of a fundoscopic criteria, or for that matter any other single criterion, for assessing the deleterious effects of laser irradiation is inadequate in producing standards which will adequately protect personnel in the field. What is needed for the establishment of realistic standards is the correlation of data from many different approaches each with its own unique assessment sensitivities to the damage process. It is nevertheless obvious from these studies that more investigation of this important environmental hazard is necessary and that modifications in the MPE should be made.

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